BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

EP 12443 (1)

(12) UK Patent Application (19) GB (11) 2 095 994 A

(21) Application No 8206489
(22) Date of filing 5 Mar 1982
(30) Priority data
(31) 56/032705
57/004222
(32) 6 Mar 1981
14 Jan 1982
(33) Japan (JP)
(43) Application published
13 Oct 1982
(51) INT CL³
A61K 9/00
(52) Domestic classification
A5B 823 829 832 M
(56) Documents cited
GB 1322482
(58) Field of search
A5B
(71) Applicants
Toyo Jozo Company Ltd.,
632-1, Ohitocho Mifuku,
Tagata-gun,
Shizouka,
Japan.
(72) Inventors
Shigeo Katsuragi,
Kenichi Sugimoto,
Yoshiaki Yamamoto,
Masataka Morishita,
Renji Aikawa,
Kazuyoshi Nishiie.
(74) Agents
Langner Parry,
52-54, High Holborn,
London, WC1V 6RR.

(54) Preparation having excellent absorption property

(57) A preparation containing an absorption promoter selected from N-acyl amino acid derivatives or N-acyl peptide derivatives represented by the formula: R-CO-A (R is an aliphatic hydrocarbon group, an aromatic hydrocarbon group or an aryl-substituted lower hydrocarbon group which may optionally be substituted, and A is an amino acid residue or a peptide residue), preferably in the presense of a salt e.g. NaCl at a concentration exhibiting higher osmotic pressure than isotonic sodium chloride solution, and a medicine is found to promote absorption of the medicine through a gastrointestinal organ such as colon and rectum, and through vagina.

GB 2 095 994 A

FIG. 2

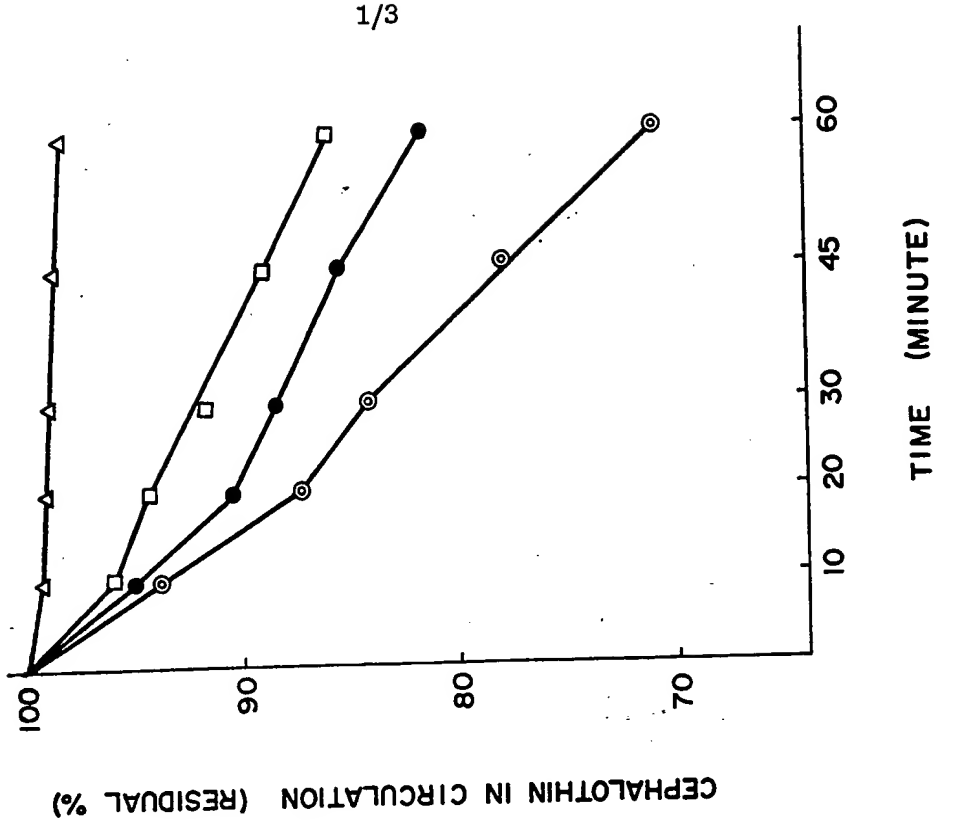

CEPHALOTHIN IN CIRCULATION (RESIDUAL %)
100
90
80
70
10
20
30
45
60
TIME (MINUTE)

FIG. 1

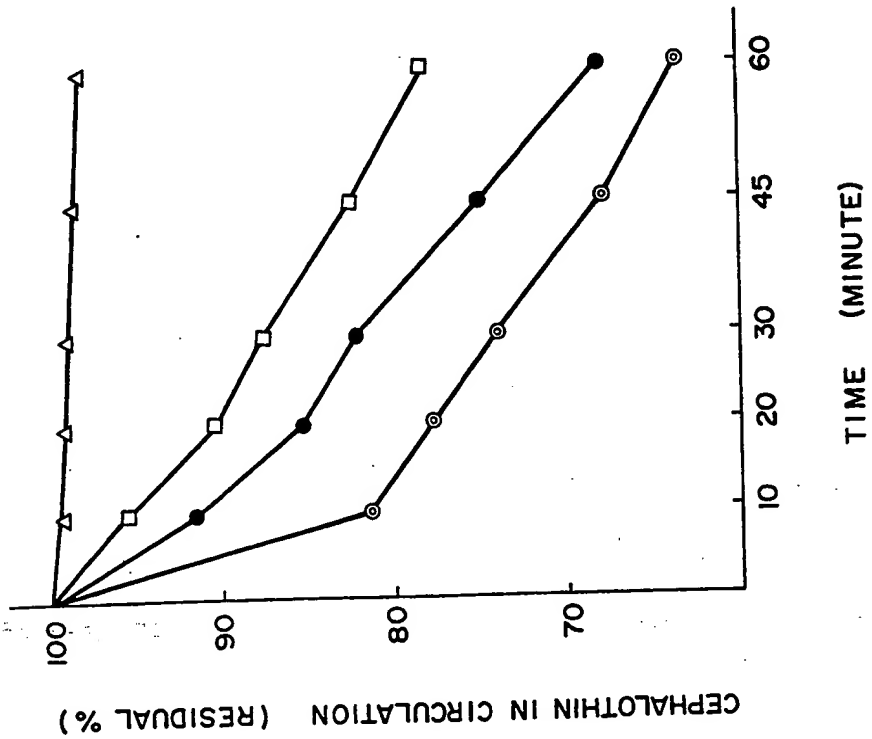

CEPHALOTHIN IN CIRCULATION (RESIDUAL %)
100
90
80
70
10
20
30
45
60
TIME (MINUTE)

FIG. 4

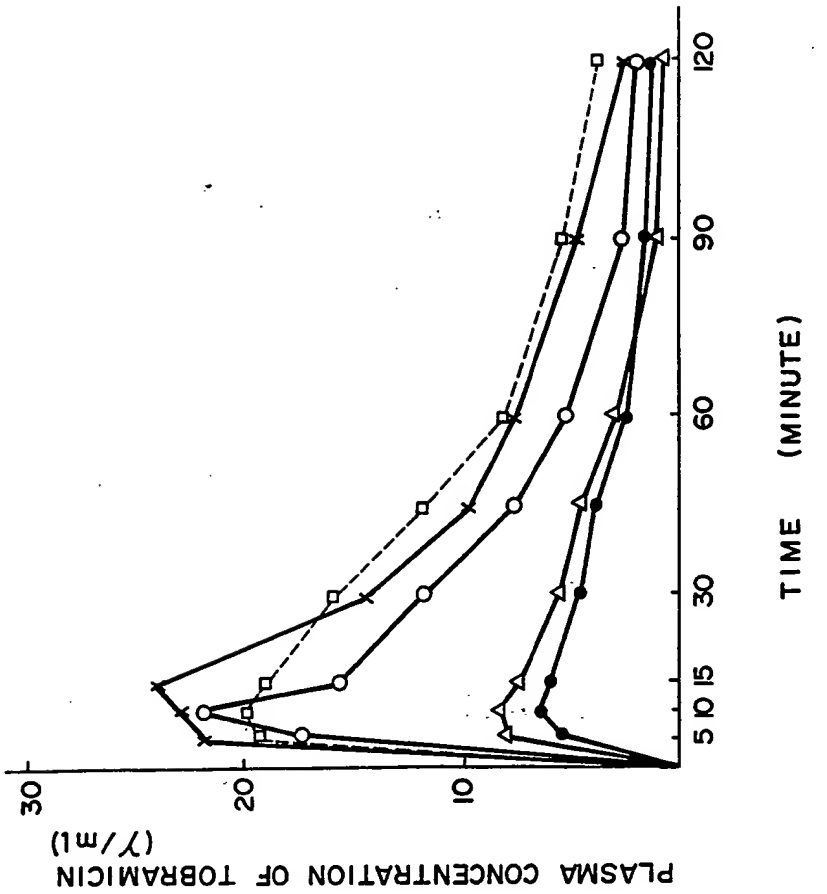
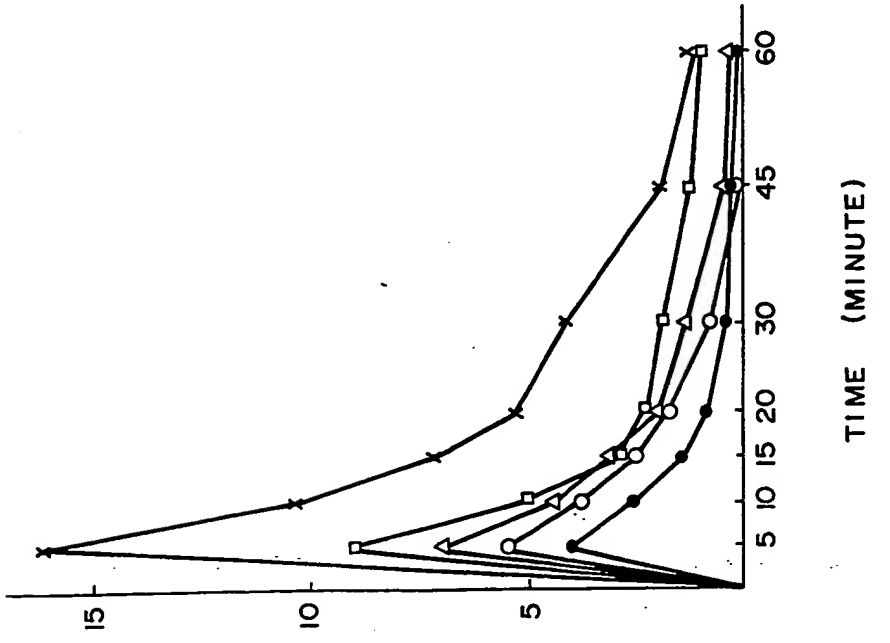

PLASMA CONCENTRATION OF TOBRAMICIN
(γ/ml)
30
20
10
5 10 15 30 60 90 120
TIME (MINUTE)

FIG. 3


PLASMA CONCENTRATION OF CEPHALOTHIN (γ/ml)
15
10
5
5 10 15 20 30 45 60
TIME (MINUTE)

2095994

FIG.6

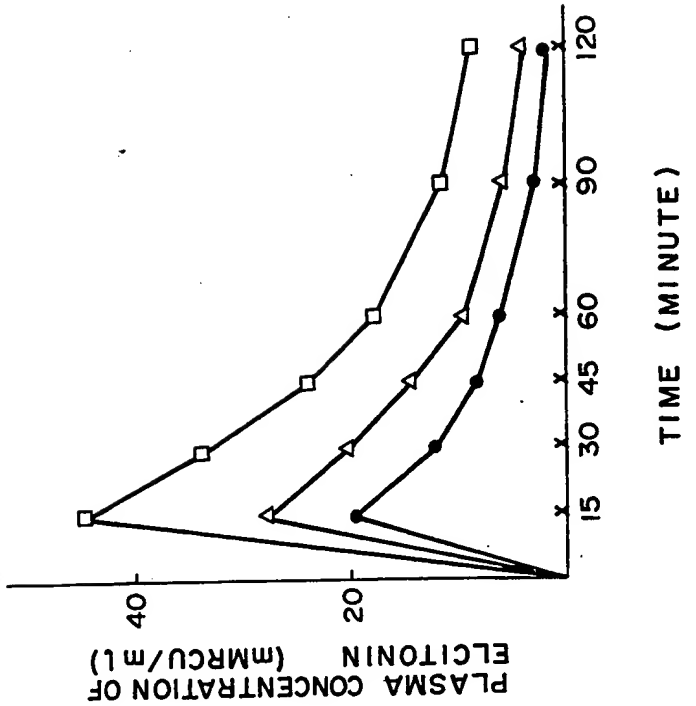
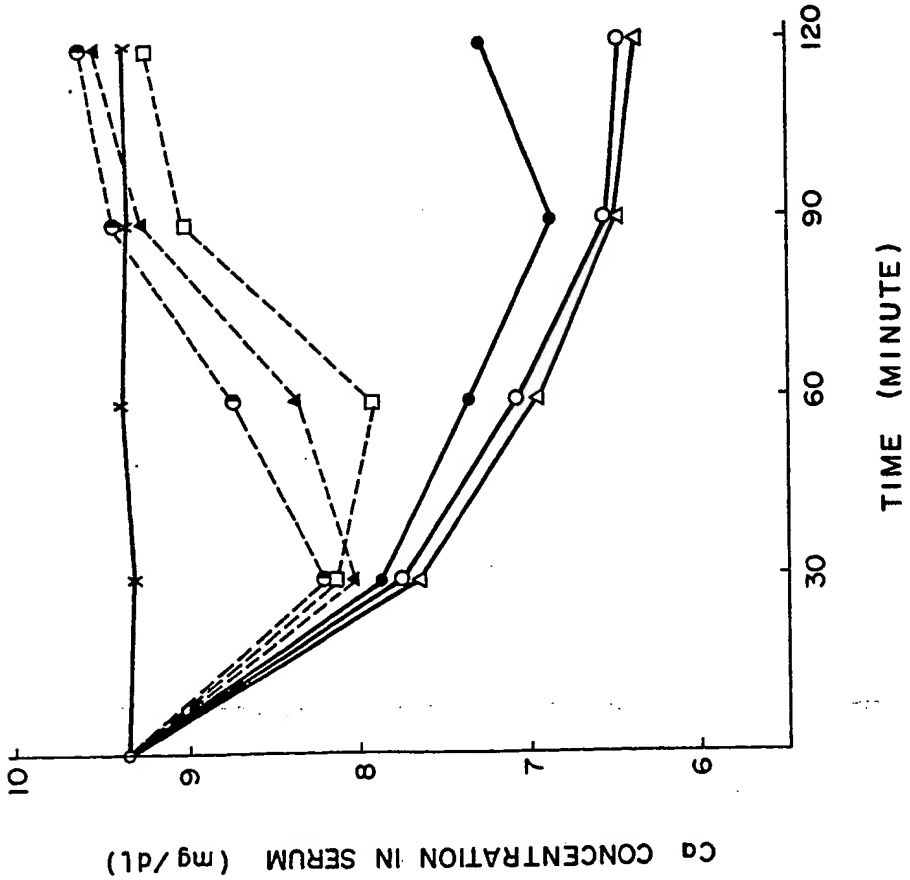


FIG.5



SPECIFICATION

Preparation having excellent absorption property

This invention relates to a novel preparation having excellent absorption property which is intended for improvement of absorption of a medicine poor in absorption property through rectum or other digestive organs in a body by administration of such a medicine simultaneously with a water-soluble substance at a concentration exhibiting higher osmotic pressure than isotonic sodium chloride solution and one or more of an absorption promoter selected from N-acyl aminoacid derivatives or N-acyl peptide derivatives as represented by the formula: R-CO-A (1), (wherein R is an aliphatic hydrocarbon group, an aromatic hydrocarbon group, an aryl-substituted lower hydrocarbon group which may optionally be substituted and A is an amino acid residue or a peptide residue).

Absorption of a medicine through a digestive organ, irrespective of whether it may be stomach, small intestine, large intestine, rectum or mouth, has heretofore been generally believed to proceed according to pH Partition theory (Modern Pharmaceutics, Marcel Dekker, INC,. p. 31 - 49). Hence, a medicine readily dissociated in respective organs at absorption sites or a medicine having poor lipophilicity tends to be poorly absorbed. Such difficultly absorptive medicines are administered as injections under the present circumstances. For improvement of absorption property of a medicine, there have been made various investigations such as Prodrug, Sofdrug, utilization of ion pairs or complex formation. But any of these proposals is effective specifically for individual medicines, and no universally applicable method is known in the art ("Pharmaceutics" written by Nogami).

The present inventors have made various investigations and consequently found that in the mechanism of membrane absorption through digestive organs or others, which is believed to proceed according to the pH partitions theory as mentioned above, an N-acyl aminoacid derivative or an N-acyl peptide derivative which is represented comprehensively by the above formula (1) causes some changes in membrane permeability, whereby membrane absorption of a medicine can be improved to promote successfully absorption thereof. Further, it has also been found that membrane absorption can be markedly improved by addition of a water-soluble substance at a concentration exhibiting higher osmotic pressure than isotonic sodium chloride solution to make the preparation under condition of higher tonicity than the osmotic pressure of a body fuild. In addition to these findings, it has further been found that a preparation obtained by use of a vehicle, additives selected as desired and an objective medicine, for example, a suppository to be inserted into rectum or vagina is a good suppository which can excellently be absorbed through membranes and maintain a high concentration of the medicine in blood for a long time. The medicines to be used in the present invention are very broad. In particular, so called water-soluble medicines having good solubility in water, for example, those with partition coefficients of 50 or less in chloroform/water or medicines readily dissociated into ions, are useful. Further, medicines applicable only as injections in the prior art are also found to be made excellently absorbable easily as preparations such as suppositories. Even a medicine with a high molecular weight such as polypeptide is also found as the result of this invention to be made efficiently absorbable in the form of a preparation such as suppository.

The present invention has been accomplished based on the above findings, and the object of the present invention is to provide a good preparation in which a medicine can be improved to have a markedly enhanced absorption property.

In the accompanying drawings,

Figures 1 and *2* show disappearance curves for various osmotic pressures of Cephalothin Na when using Cephalothin Na as medicine, in which the percentages of Cephalothin disappeared by absorption are plotted at various measurement time;

Figure 3 a curve of plasma concentration of Cephalothin when using Cephalothin Na as medicine;

Figure 4 a curve of plasma concentration of Tobramicin when using Tobramicin as medicine;

Figure 5 a curve of calcium concentration in serum when using Elcitonin as medicine; and

Figure 6 a curve of plasma concentration of Elcitonin when using Elcitonin as medicine.

According to the present invention, a preparation is provided which comprises a water-soluble substance at a concentration exhibiting an osmotic pressure higher than isotonic sodium chloride solution, at least one absorption promoter selected from the group consisting of N-acyl amino acid derivatives or N-acyl peptide derivatives represented by the formula: R-CO-A (1) (where R and A are the same as those defined above) and a medicine.

To speak first of a water-soluble substance to be used in the present invention at a concentration exhibiting higher osmotic pressure than isotonic sodium chloride solution, it may be preferably one which is harmless as a whole and can exhibit high osmotic pressure with a small amount as possible.

As such a water-soluble substance, there may be included water-soluble salts and water-soluble sugars.

Particularly among water-soluble salts, sodium chloride is preferred since it is safe and readily controllable of its osmotic pressure, and further soluble in water rapidly at a high dissolving rate. Further, mannitol or glucose is preferred among water-soluble sugars, Generally speaking, water-soluble salts may include, for example, halides, sulfates, phosphates or carbonates of alkali metals such as sodium, potassium or lithium, more specifically the aforesaid sodium chloride, sodium sulfate, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium phosphate, sodium hydrogen carbonate, sodium carbonate, potassium

chloride, potassium sulfate, potassium hydrogen phosphate, potassium carbonate, lithium chloride, etc. These salts may be adjusted to concentrations exhibiting higher tonicity than osmotic pressure of isotonic sodium chloride solution depending on the osmotic characteristic thereof. For example, in case of sodium chloride, it may generally be adjusted to a concentration of 1 W/W% or higher of the whole content. The upper limit of the concentration is not particularly limited, but preferably the concentration is about 2 to 30 W/W%. As preferable water-soluble sugars, there may be employed monosaccharides or disaccharides frequently used for adjustment of osmotic pressure in pharmaceutical technology, including, for example, glucose, mannitol, sorbitol, xylitol, lactose, maltose and sucrose. Such a sugar may be used at a concentration with higher tonicity than isotonic sodium chloride solution, which is generally 0.25 M or higher. These water-soluble substances may be used in combination of two or more kinds for adjustment of osmotic pressure, which is preferably 1.5 to 6-fold of the osmotic pressure exhibited by isotonic sodium chloride solution.

In connection with osmotic pressure, description is herein made by comparison with isotonic sodium chloride solution, but such description with the use of isotonic sodium chloride solution as Control is merely exemplary for comparison between osmotic pressure, and therefore may also be possible with the use of body fluids or other solutions of salts with tonicity equal to such isotonic sodium chloride solution.

Referring now to the N-acyl aminoacid derivatives or N-acyl peptide derivatives which are represented by the formula R-CO-A (1) (R and A are same as those defined above) to be used as an absorption promoter in this invention, they were investigated by adding to, for example, isotonic preparation for rectal application containing a medicine for examination of increase or decrease of membrane permeability of the medicine to accomplish the present invention. The mechanism of the promotion effect has not so far been clarified, but it seems likely that membrane absorption mechanism may be changed through the chelating action and affinity to membrane possessed by these absorption promoters on the structures of cell membranes or the spaces between the epitherial cells thereby to promote absorption.

Although the mechanism action of the absorption promoter for increase of drug absorption through rectum or other organs may be speculated as mentioned above, such a mechanism action is still no more than mere estimation and it is only sufficient to employ N-acyl aminoacid derivatives or N-acyl peptide derivatives having chelating action capable of bonding to at least calcium ions or magnesium ions.

Further, referring to the N-acyl amino acid derivatives or N-acyl peptide derivatives of the formula (1), these compounds are obtained in this invention by reacting an acid compound of the formula:

$$R-COOH \qquad (2)$$

(wherein R is the same as that defined above)
or its reactive derivative of carboxyl group with a compound of formula (3):

$$H-A \qquad (3)$$

(wherein H—A is an amino acid or a peptide)
or its derivative having protected carboxyl group. Alternatively, the compound of the formula (1) may also be obtained by condensation of an acyl group of the above acid compound (2) and the amino residue group of the imino residue group of an amino acid or a peptide.

Carboxyl groups can be activated by agents such as an acid azide, an acid anhydride, an acid imidazolide, an acid halogenide, an active ester or a carbodiimide, an N,N'-carbonyl-diimidazole or an isoxazolium salt such as Woodward's reagent.

The preferred condensation reaction in the present invention is the carbodiimide, azide, active ester, halogenide or anhydride method.

As example of condensed reaction, amino acid or peptide is reacted with reactive derivatives of acid-compound of formula (2) in an inert medium, for example, an organic solvent such as chloroform, methylenechloride, ethylenechloride, ether and hydrophilic solvent or an aqueous hydrophilic solvent such as water, methanol, ethanol, acetone, dimethylacetoamide, dimethylformamide and dioxane. N-acylation is performed by usual method, and if necessary, a condensation agent or a base may be added to the above solvent.

In this reaction, reaction temperature is below or at room temperature, and the acid compound of formula (2) is used about one mole per mole of amino acid or peptide. After the reaction, it may be purified by preferably, a method of gel filtration.

As an acid-compound of the formula (2), there may preferably be used an acid in which R is an aliphatic hydrocarbon group which may be substituted or an aromatic hydrocarbon group which may be substituted or an aryl-substituted lower aliphatic acid in which carbon atoms of the lower aliphatic acid group generally have in the range of 1 to 10, preferably 1 to 5, which may be substituted.

When R is an aliphatic hydrocarbon group which may be substituted, the hydrocarbon group may have

carbon atoms generally in the range of from 1 to 20, preferably 4 to 18. As aliphatic acids having such groups, there may be mentioned, for example, acetic acid, propionic acid, butyric acid, valeric acid, iso-valeric acid, hexanoic acid, enathic acid, octanoic acid, pelargonic acid, decanoic acid, undecylic acid, lauric acid, myristic acid, palmitic acid, stearic acid, acrylic acid, crotonic acid, vinylacetic acid, 4-pentenoic acid, oleic acid, linoleic acid, linolenic acid or sorbic acid as fatty acid and these compounds substituted with a lower alkyl group, hydroxyl group, carboxylic acid group, alkoxy group, phosphoric acid group, amino group, imino group or a halogen.

As acids wherein R is an aromatic hydrocarbon group which may be substituted, there may be mentioned, for example, benzoic acid, alkylbenzoic acid, phthalic acid, isophthalic acid, o–, m– or p-aminobenzoic acid, o–, m– or p-hydroxybenzoic acid, o–, m– or p-alkoxybenzoic acid, dihydroxybenzoic acid and these compounds substituted with above functional groups.

Acids wherein R is an aryl-lower-hydrocarbon group which may be substituted may include, for example, phenylacetic acid, phenylpropionic acid, o–, m– or p-hydroxy phenylacetic acid, phenylmalonic acid, phenylsuccinic acid, cinnamic acid, phenyl-pyruvic acid, benzoylacetic acid and these compounds substituted with above functional groups.

As an amino acid or a peptide of the formula (3), natural amino acid or peptide may preferably be used. They can be in either D–, L– or DL-form, and the derivatives thereof substituted with hydroxyl, amino, carboxylic acid, lower hydrocarbon, alkoxy, phosphoric acid or a halogen atom as well as ethyl or methyl ester derivatives thereof are also available. There may be mentioned, for example, various natural amino acids (Chemistry of Amino Acids, Vol. 1, p. 3-8 etc.) o–, m– or p-hydroxy phenylalanine, o–, m– or p-hydroxy phenylglycine, α–, β– or γ– carboxy glutamic acid, glutamic acid α-methylester, glutamic acid α-ethylester, aspartic acid α-ethylester, α–, β– or γ-amino butyric acid, iminodiacetic acid and others.

As the peptide to be acylated, there may generally be used a peptide composed of two or more amino acids, preferably of 2-4 amino acids, which can be obtained by alkali, acid or protease hydrolysis of natural protein such as collagen, keratin, fibroin, albumin, globulin, gelatin and also by conventional synthetic methods. These N-acyl amino acid derivatives or N-acyl peptide derivatives preferably posses a chelating activity of about one-thousandth or more of EDTA (ethylenediaminetetraacetic acid).

These N-acyl amino acid derivatives or N-acyl peptide derivatives mentioned above are preferably used in the form of alkali metal salts such as sodium salts, potassium salts or ammonium salts.

An N-acyl amino acid derivative or an N-acyl peptide derivative which is represented by the above formula (1) is used in the present invention as absorption promoter.

These absorption promoters may be employed in amounts of 0.01 W/W% or more, generally in the range of from 0.1 to 30 W/W%, preferably from 1.0 to 20 W/W%. As the vehicle to be employed for preparation of a suppository containing the above absorption promoter, a medicine and preferably a water-soluble salt to be added for increase of tonicity, there may suitably be selected one from oily vehicles and water-soluble vehicles conventionally used in preparation of suppositories or rectal injections, and a surfactant may also be added if desired.

As these oily vehicles or water-soluble vehicles, there may conveniently be used those as described in "The Theory and Practice of Industrial Pharmacy", p. 245 to 269 (1976).

The medicine to be used in the present invention is not particularly limited, but there may be employed ordinary pharmaceuticals, particularly preferably so called water-soluble medicines which are excellently soluble in water, such as water-soluble medicines with a partition coefficient of 50 or less in chloroform/water or medicines readily dissociated to ions. For example, there may be included various medicines such as hypnotics, tranquilizers, antiepileptics, antipyretics, antalgics, antidepressants, muscle relaxants, antiinflammatory agents, antiallergic agents, immunosuppressants, antirheumatics, vasodilators, antihemorragics, antihypertensives, antibiotics, antibacterial agents, urinary tract sterilizers, antitumor agents, vitamins, hormones and galenicals. More specifically, typical examples are penicillin type antibiotics such as ampicillin, hetacillin, amoxicillin, cyclacillin, cloxacillin, dicloxacillin, oxacillin, carindacillin, sulbenicillin, piperacillin, apalcillin, methycillin, etc. or combined drugs of ampicillin or amoxicillin with oxacillin, cloxacillin, floxacillin or dicloxacillin; cephalosporin type antibiotics such as cephalothin, cephazolin, cephaloridine, cephacetorile, cefoxitin, cefadroxil, cefatridine, cephaloglycin, cephalexin, cephapirin, cephachlor, ceftexol, cefuroxime, cefsulodin, cefmetazole, etc. and non-toxic salts thereof such as alkali metal salts (e.g. sodium salts or potassium salts), ammonium salts or benzylamine salts. In addition, there may also be mentioned tetracycline type antibiotics such as doxyclycline, oxycycline, etc; aminosaccharide type antibiotics such as kanamycin, sisomicin, amikacin, tobramycin, netromycin, gentamycin, 1–N–(s–3–amino–2-hydroxypropionyl)-gentamicin B, etc.; peptide type antibiotice such as tuberactinomycin N, actinomycin, etc. or non-toxic salts thereof; futher peptide hormones such as insulin, somatostatin, calcitonin, angiotensin, kallikrein, secretin, gastrisin, parathyroid hormone, etc.; and other medicines such as barbital, theophylline, aspirin, mizoribin (bredinine), 5-fluorouracil, methotrexate, L-dopa, etc. The medicine may be employed in an amount, which may suitably be selected and designed. For example, in case of antibiotics such as β-lactam antibiotics, 20 to 500 mg activity, generally 100 to 300 mg activity, or in case of peptide hormones such as insulin, 1 to 500 units may be contained per gram of preparation. In general, the medicine may preferably be used in finely divided forms with 1 to 50μ diameters or as an aqueous solution.

The step of forming preparations may be performed according to conventional methods for production of

preparations in general such as rectal suppository, urethral suppository or vaginal suppository, ointments or creams. For example, the absorption promoter selected, a water-soluble substance in an amount exhibiting higher osmotic pressure than isotonic sodium chloride solution and a medicine are added to a vehicles, optionally in combination with a surfactant, and these components are thoroughly mixed to provide preparations.

Futher, in production of these preparation, there may also be added preservatives such as methyl- or propyl-p-oxybenzoate, colorants, aromas and stabilizers.

The present invention is further illustrated in detail by referring to the following Examples, by which the present invention is not limited at all but various medicines, hypertonicators and absorption promoters may be selected and combined in addition to those shown in Examples.

The abbreviations of employed in Examples are as follows,

Gly:	Glycine
Pro:	Proline
Phe:	Phenyl alanine
Asp:	Aspartic acid
Glu:	Glutamic acid
Val:	Valine
Thr:	Threonine
Ile:	Isoleucine
Phy:	Phenyl glycine
Lys:	Lysine
ε-Acp:	ε-Aminocaproic acid
OEt:	Ethyl ester
OMe:	Methyl ester
Boc:	t-Butyloxy carbonyl
HOBt:	1-Hydroxy benzotriazole
TFA:	Trifluoroacetic acid
DMF:	Dimethyl formamide
WSCD:	N-Ethyl, N-3-dimethylaminopropyl carbodiimide
AcOEt:	Ethyl acetate
NMM:	N-Methyl morpholine
EtOH:	Ethanol
HCl:	Hydrochloric acid
NaOH:	Sodium Hydroxide
TMA:	Trimethyl amine

Example 1

Absorption effects under conditions with various tonicities were examined. Each sample solution was prepared by adding 0.1 W/V% cephalothin Na as a medicine together with 0.01 W/V% N-lauroyl Gly-ONa as an absorption promoter to a phosphate buffer of pH 7.5 conditioned with sodium chloride to a tonicity which was varied from isotonic to twice hypertonic than isotonic (two-fold tonicity), four times hypertonic than isotonic (four-fold tonicity).

The experiment was conducted according to the following method. Namely, Wistar-strain male rats, weighing 250 to 300 g, were narcotized (after fast for 20 hours) with pentobarbital (50 mg/kg) and thereafter subjected to hypoabdominal incision for a first cannulation at a position about 1.5 cm from anus and also annother cannulation at a position 5 cm upper than said first cannulation. Subsequently, rectum was internally washed with about 20 ml isotonic sodium chloride solution kept at 38°C, and samples each of 10 ml were circulated through rectum for 5 minutes (2 ml/minute) to make the concentration in the system constant. Then, 5 ml of each sample was circulated at a flow rate of 2 ml/minute, and samples each of 0.05 ml were collected at intervals of 10 minutes from 0 minute. Each sample was diluted to 5 ml with distilled water and the quantity of medicine disappeared by absorption was determined by UV-spectro photometer.

As the result, the disappearance curve of Cephalothin-Na under the condition of 0.01 W/V% N-lauroyl Gly-ONa was obtained as shown in Figure 1, in which □–□ shows the result under the isotonic condition, ● – ● under two-fold tonicity, ◎ – ◎ under four-fold tonicity and △ – △ under no absorption promoter (Control).

Example 2

Using 0.1 W/V% cephalothin Na as a medicine and 0.1 W/V% of N-myristoyl Pro-Pro-GlyNa as an absorption promoter under respective osmotic pressure conditions (namely isotonic, two fold tonicity and four fold tonicity with the use of sodium chloride) and following otherwise the same procedure as in Examrple 1, quantities of Cephalothin disappeared by absorption were determined. The results are shown in Figure 2, in which□–□ shows the result under the isotonic condition, ● – ● under two-fold tonicity, ◎ – ◎ under four-tonicity and △ – △ Control.

Example 3

Quantities of 0.1 W/V% Cephalothin Na disappeared by absorption under isotonic and two-fold tonic and four-fold tonic conditions were determined, respectively, using N-acyl amino acids and N-acyl peptides where the N-acyl groups are aliphatic hydrocarbons, similarly as in Example 1. The results are shown in Table 1.

TABLE 1
(values after 60 minutes)

	Isotonic condition	*Two-fold tonic condition*	*Four-fold tonic condition*
N-Propionoyl PheONa	7.3%	-%	19.2%
N-Hexanoyl PheONa	13.5	20.9	37.6
N-Octanoyl PheONa	22.5	31.7	42.1
N-Octanoyl PheOH	12.5	-	24.5
N-Hexanoyl ValONa	4.7	-	16.2
N-Hexanoyl GlyONa	18.6	25.8	34.8
N-Octanoyl GlyONa	20.5	26.4	34.7
N-Decanoyl GlyONa	19.5	24.6	36.2
N-Myristoyl GlyONa	10.1	-	20.4
N-Hexanoyl AspONa	21.0	-	34.0
N-Octanoyl AspONa	19.5	-	31.0
N-Decanoyl AspONa	16.9	-	26.4
N-Lauroyl AspONa	21.8	35.8	47.4
N-Myristoyl GlyONa	10.3	15.7	20.7
N-Decanoyl Pro-Pro-GlyONa	7.8	14.0	20.3
N-Lauroyl Pro-Pro-GlyONa	12.4	-	22.4
N-Myristoyl Pro-GlyONa	9.5	15.4	20.6
No adjuvant	2.1	3.3	6.2

Example 4

Quantities of 0.1 W/V% Cephalothin.Na disappeared by absorption under isotonic, two-fold tonic and four-fold tonic conditions were determined by the high pressure liquid chromatography respectively, using N-acyl amino acids and N-acyl peptide in which the N-acyl groups are aromatic hydrocarbons and aryl-substituted low hydrocarbons, similarly as in Example 1. The result are shown in Table 2.

TABLE 2
(values after 60 minutes)

	Isotonic condition	*Two-fold tonic condition*	*Four-fold tonic condition*
N-Benzoyl AlaONa	22.6%	30.1%	39.8%
N-Benzoyl ThrONa	23.5	28.7	40.0
N-Benzoyl ε-AcpONa	20.7	32.3	37.7
N-Phenacetyl LysONa	7.6	-	17.8
N-Phenacetyl IleONa	11.8	-	21.9
p-Aminobenzoyl GlyONa	-	16.2	22.5
N-Phthaloyl GluONa	24.1	27.3	30.4
N-Phthaloyl Glu α-methyl ester	22.7	-	32.4
N-Phthaloyl IleONa	23.1	30.4	35.1
N-Benzoyl GlyAspONa	12.7	-	25.1

Example 5

Cephalothin.Na (600 mg potency) as a medicine, N-acyl peptides (100 mg) as an absorption promoter and sodium chloride (200 mg) as a hypertonicator were each pulverized and dispersed in 1 ml of distilled water. A homogeneous dispersion was prepared by adding the resulting mixture to a base of Witepsol H-15 previously molten by fusion to a total amount of 10 g. The dispersion was intrarectally administered at a dose of 30 mg/kg to Wister strain rats (male, weighing 200-250 g, four per one group) and blood sampling was performed 5 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes, 45 minutes and 60 minutes after administration for measurement of Cephalothin concentration in plasma (according to the bioassay using Bacillus subtilis ATCC 6633). As Controls, there was also obtained a preparation without use of the absorption promoter (Control 1). N-Acyl peptides as the absorption promoter used were N-Myristoil Pro-Pro-GlyONa, N-Lauroyl Pro-Pro-GlyONa, N-Decanoyl Pro-Pro-GlyONa and N-Myristoyl Pro-GlyONa. The result are shown in Figure 3, wherein ● – ● indicates plasma concentration curve of Cephalothin in the case of control, □ – □ that in case of N-Myristoyl Pro-Pro-GlyONa as the absorption promoter, ○ – ○ that in case of N-Lauroyl Pro-Pro-GlyONa, △ – △ that in case of N-Decanoyl Pro-Pro-GlyONa and x – x that in case of N-Myristoyl Pro-GlyONa, respectively.

Example 6

Tobramycin (200 mg potency) as a medicine, N-acyl amino acids (10 mg) as a absorption promoter and sodium chloride (200 mg) as a hypertonicator were each pulverized and mixed together. The mixture was mixed with Witepsol H-15 molten by heating to an amount of 1 g. The dispersion was intrarectally administered at a dose of 20 mg^{p}/kg and experiment was carried out similarly as in Example 5. As Control, there was also obtained a preparation without use of the absorption promoter. N-acyl aminoacids as the absorption promoters used were N-Lauroyl PheONa, N-Butyryl PheONa, N-Lauroyl PhyONa and N-Butyryl PhyNa. The result are shown in Figure 4, wherein ● – ● indicates plasma concentration curve of Tobramycin in the case of Control, △ – △ that in case of N-Butyryl PheONa, ○ – ○ that in case of N-Butyryl PhyONa, □ – □ that in case of N-Lauroyl PheONa and x – x N-Lauroyl PhyONa, respectively.

Example 7

Elcitonin (Asu^{1,7}–eel calcitonin) (10 units and 2 units), N-acyl amino acids, N-acyl peptide and sodium chloride (50 mg) were dissolved in 1 ml of 5% gelatin solution. Each solution (0.1 ml) was administered intrarectally to SD-strain male rats (four weeks of age) and calcium concentrations in serum were measured at 30 minutes, 60 minutes, 90 minutes and 120 minutes after administration by atomic absorption method. As Control, there was used a solution containing no absorption promoter (adjusted to 10 units of Elcitonin). N-acyl amino acids and N-acyl peptide as the absorption promoter used were N-Lauroyl GlyONa, N-Hexanoyl PheOH, N-Myristoyl Pro-Pro-GlyONa and N-Lauroyl AspONa. The results are shown in Figure 5, wherein x – x indicates calcium concentration in serum in case of Control, ▲ – ▲ that in case of solution containing 2 units of Elcitonin and N-hexanoyl PheONa, ◑ – ◑ that in case of 2 units of Elcitonin and N-Lauroyl AspONa, □ – □ that in case of 2 units of Elcitonin and N-Lauroyl GlyONa, ● – ● that in case of 10 units of Elcitonin and N-Myristoyl Pro-Pro-GlyONa, ○ – ○ that in case of 10 units of Elcitonin and N-Hexanoyl PheOH and △ – △ that in case of 10 units of Elcitonin with N-Lauroyl GlyONa.

Example 8

Elcitonin (Asu^{1,7}–eel calcitonin) of 200 units, N-acyl amino acids as the absorption promoter (50 mg) and sodium chloride (50 mg) were dissolved in 1 ml of a 5 % gelatin solution. Each solution (0.1 ml) was administered intrarectally to Wister-strain rats (200-250 g) and Elcitonin concentration in plasma were determined by enzyme-immunoassay method at 15, 30, 45, 60, 90 and 120 minutes after administration. N-acyl amino acids as the absorption promoter used were N-Octanoyl GlyONa, N-Octanoyl PheONa and N-Lauroyl GlyONa. As Control, there was used a solution containing no absorption promoter. The results are shown in Figure 6, wherein □ – □ indicates Elcitonin concentration in plasma in case of N-Octanoyl PheONa, △ – △ that in case of N-Octanoyl GlyONa, ● – ● that in case of N-Lauroyl GlyONa and x – x that in case of Control. Further, Elcitonin concentration was not detectable in case of Control.

Example 9

The collagen was hydrolyzed in 0.1 N-HCl, then fractionation performed using a column of Sephadex G-25 and fractions of molecular weight in the region of 900 were collcted. The equimolar quantity of the above peptide was reacted with each acid chloride of hexanolic acid, decanolic acid, lauric acid and stearic acid in DMF containing TMA in an ice-bath, to give each hydrolyzed product of N-acyl Collagen. These N-acyl peptides were dissolved in equimolar quantities of 1N-NaOH solution, followed by concentration and, freeze-drying to obtain sodium salts of these N-acyl peptides, respectively.

1. Using N-lauroyl derivative of hydrolyzed product of collagen as an absorption promoter, each rectal suppository containing 0.1 W/W%, 2.5 W/W%, 5 W/W% and 7.5 W/W% of the absorption promoter together with ampicillin·Na as a medicine and Witepsol H-15 as a suppository base. These preparations were intrarectally administered at a dose of 15 mg/kg to male rabbits and ampicillin concentrations in plasma were assayed according to the bioassay method using ATCC 6633.

As the result, peak plasma concentrations of ampicillin were 1.4 γ/ml (no absorption promoter), 2.5 γ/ml

(addition of 0.1 W/W%), 4.4 γ/ml (addition of 2.5 W/W%), 7.0 γ/ml (addition of 5.0 W/W%) and 5.8 γ/ml (addition of 7.5 W/W%), respectively. From the result, it can be seen that the aborption promoters may preferably be employed in the range of from 0.1 W/W% to 5 W/W%.

2. The rectal suppository containing each 5 W/W% of N-acyl derivative of hydrolyzed product of collagen together with ampicillin·Na as a medicine and Witepsol as a base. Then the samples were administered intrarectually each at a dose of 15 mg/kg to male rabbits and the concentrations of ampicillin in plasma were assayed. The results are shown in Table 3. From the result, it can be seen that N-stearoyl derivative of hydrolyzed product of collagen is preferably effective. Further, using 0.5 W/W% of N-stearoyl derivative of hydrolyzed product of collagen, other β-lactam antibiotics such as cephalothin·Na, cephazolin·Na and cephapilin·Na were examined under the same conditions as mentioned above. As the result, the peak concentrations of cephalothin was 12.8 γ/ml, that of cephazolin 35.8 γ/ml and that of cephapilin 19.0 γ/ml, respectively. On the other hand, in the case of no absorption promoter, the concentration was not detectable for cephalothin and cephazolin, or 4.5 γ/ml for cephapilin.

TABLE 3

Chain length of fatty acid	*Plasma level (γ/ml) Time (min.)*					
	5	*10*	*20*	*40*	*60*	*90*
C_{18}	3.0	8.0	10.2	5.0	2.9	1.9
C_{12}	5.2	6.8	5.3	3.1	1.8	0.79
C_{10}	6.7	7.2	4.4	1.6	0.63	-
C_8	4.8	5.1	3.4	1.5	0.61	-
No addition	1.1	1.2	0.95	0.68	0.52	-

3. And the next, absorption effects under conditions with various tonicities were examined as follows. Each sample was prepared by adding 5 mg/ml of ampicillin·Na as a medicine together with 5 W/W% or 0.1 W/W% of N-stearoyl derivative of hydrolyzed product of collagen peptides and with 1.7 W/W% of NaCl (twice hypertonic condition), 10 W/W% of glucose (twice hypertonic condition) or 0.85 W/W% of NaCl (isotonic condition). The experiment was carried out using Recirculating Perfusion Method (Wistar strain rats). The plasma concentrations were determined during recirculation of 10 ml each of above solution and the results are shown in Table 4.

TABLE 4

	N-acyl derivative of hydrolyzed product of collagen	*Plasma level (γ/ml) Time (min)*				
		10	*20*	*40*	*60*	*90*
NaCl 1.7W/W%	5.0 W/W%	4.5	32.7	59.2	32.5	20.2
	0.1W/W%	3.7	28.5	45.0	25.6	14.3
Glucose 10 W/W%	5.0 W/W%	5.2	33.9	54.6	34.4	25.1
	0.1 W/W%	3.0	25.7	43.8	30.2	21.5
NaCl 0.85W/W%	5.0 W/W%	3.9	7.3	14.9	11.6	10.0
	0.1 W/W%	3.2	4.4	7.6	6.3	4.2

4. The experiment was performed similarly as in Example 9-3 except that N-decanoyl collagen peptide was used as an absorption promoter instead of N-stealoyl collagen peptide. The results are shown in Table 5.

From the results shown in Tables 4 and 5, it may be concluded that the absorption of ampicillin Na can be improved to promote successfully under the hypertonic conditions by addition of NaCl or saccharide. Further, similar results were obtained for other β-lactam antibiotics such as cephalothin Na, cephazolin Na and cephapiline Na.

TABLE 5

	Plasma level (γ/ml)				
	Time (min.)				
	10	*20*	*40*	*60*	*90*
NaCl 1.7 W/W%	3.9	22.7	32.9	16.0	8.1
Glucose 10 W/W%	3.6	20.2	34.2	17.5	10.8
NaCl 0.85 W/W%	2.6	6.6	8.5	7.4	6.9

Example 10

Cephalothin·Na (200 g potency), N-lauroyl GlyONa (50 g) and sodium chloride (50 g), each being pulverized, were mixed and the resulting mixture was dissolved in 2 % gelatin solution to a volume of one liter, which was then filled into injection cylinders in aliquots each of 1 ml to provide intrarectal injection preparations.

Example 11

Gentamycin (100 g potency), N-Decanoyl AspONa (50 g) and mannitol (250 g), each being pulverized, were mixed and the mixture was homogeneously dispersed in 5 % gelatin solution to a volume of one liter, which was then filled into injection cylinders in aliquots each of 1 ml to provide intrarectal injection preparations.

Example 12

One thousand units of Elcitonin, 50 g of N-Hexanoyl PheONa and 250 g of mannitol were each pulverized and mixed together. The resulting mixture was dispersed homogeneously in 5 % gelatin solution to a volume of one liter, which was then filled into injection cylinders in aliquots each of 1 ml to provide injection preparations for vaginal suppository.

Example 13

One thousand units of Elcitonin, 50 g of N-Myristoyl Pro-GlyONa and 5 g of sodium chloride were dissolved in 100 ml of distilled water and the solution was added to Witepsol H-5 containing 1 % Span 60 (produced by Kao-Atlas Co.) to an amount of 500 g, followed further by homogeneous emulsifying. The emulsion was filled in suppository containers in aliquots each of 1 g to provide rectal suppositories.

Example 14

Cefoxitin·Na (200 g potency), N-phthaloyl GlyONa (50 g) and sodium chloride (50 g) each being pulverized were mixed and dispersed in Witepsol H-5 molten by heating to an amount of 1 kg, which was then filled in suppository containers in aliquots each of 1 g to provide suppositories.

Example 15

Example 14 was repeated except that Cefazolin·Na (200 g potency) was employed in place of Cefoxitin·Na to obtain suppositories.

Example 16

1-N-(s-3-amino-2-hydroxypropionyl) gentamycin B (100 g potency), N-Hexanoyl GlyONa (10 g) and sodium chloride (50 g) were each pulverized and mixed. The mixture was mixed and homogeneously dispersed with Witepsol H-5 molten by heating to an amount of 1 kg. The dispersion was molded in suppository containers to provide suppositories each of 1 g.

Example 17

Ampicillin Na (25.g potency), NaCl (3.4 g) and N-stearoyl derivative of hydrolyzed product of collagen (5 g), each being pulverized, were mixed and the mixture was homogeneously dispersed in Witepsol H-15 molten by heating at 45°C to an amount of 100 g. The emulsion was filled in suppository containers to provide rectal suppositories under cool conditions.

Example 18

Ampicillin Na (20 g potency), NaCl (2.5 g) and N-stearoyl derivative of hydrolyzed product of collagen, each being pulverized, were dispersed in peanut oil containing Emulgen 408 (3.5 g, Kao Atlas Co., Ltd.; polyoxyethylene oleylalcohol ether) and was made up to an amount of 100 g. Each 1.5 g of dispersion was filled in gelatin rectal capsules to provide rectal capsules.

Example 19

Ampicillin Na (25 g potency), glucose (10 g) and N-stearoyl derivative of hydrolyzed product of collagen (5 g), each being pulverized, were mixed and the mixture was homogeneously dispersed in Witepsol H-15 molten by heating to an amount of 100 g. The emulsion was filled in suppository containers to provide rectal suppositories.

Example 20

Example 19 was repeated using glucose (10.9 g) and N-stearoyl derivative of hydrolyzed product of collagen (0.1 g) in place of glucose (10 g) and N-stearoyl derivative of hydrolyzed product of collagen (5 g) to provide suppositories.

Example 21

Example 20 was repeated using NaCl (3.4 g) in place of glucose to provide suppositories.

Example 22

Example 19 was repeated using cephalothin.Na as a medicine in place of ampicilline.Na and using NaCl (3.4 g) instead of glucose to provide suppositories.

Example 23

Cephazolin-Na (20 g potency), glucose (10 g) and sodium salt of N-stearoyl derivative of hydrolyzed product of collagen (2.5 g) being pulverized were dispersed in peanut oil containing NIKKOL BC-20 TX (4.5 g, Nikko Chemical Co., Ltd.: polyoxyethylene cetylalcohol ether) to an amount of 100 g, which was filled into gelatin rectal capsules in aliquots each of 1.5 ml to provide rectal capsules.

Example 24

Example 23 was repeated except that sulbenicillin·Na (20 g) was employed in place of cephazolin·Na to provide rectal capsules.

Example 25

Example 23 was repeated except that Enviomycin·sulfate (20 g potency) was employed in place of cephazolin·Na to provide rectal capsules.

Example 26

Mannitol (10 g) and N-stearoyl derivative of hydrolyzed product of collagen (2.5 g), each being pulverized together with 12500 Units of Elcitonin were mixed and the mixture was homogeneously dispersed in Witepsol H-15 molten by heating to an amount of 100 g. Then, the emulsion was filled in suppository containers to provide rectal suppositories.

Example 27

Example 26 was repeated except that Witepsol S-55 was used instead of Witepsol H-15 and homogeneously dispersed. The emulsion was filled in vaginal suppository containers to provide each 2 g of vaginal suppositories.

Example 28

The pulverized dicloxacillin Na (5 g potency) and Mygriol 812 (13.8 g with moisture contents of 0.13 %) were mixed and the above mixed solution was added to ampicillin trihydrate (10 g potency), NaCl (3.4 g) and N-stearoyl derivative of hydrolyzed product of collagen sodium salt (2.5 g), each being pulverized, then homogeneously dispersed in Witepsol H-5 molten by heating to an amount of 100 g. The emulsion was filled into gelatin rectal capsules to provide 1 g each of rectal suppositories.

Example 29

Pulverized glucose (10g) and N-stearoyl derivative of hydrolyzed product of collagen in the form of sodium salt (5 g) and 12500 Units of Elcitonin were added to 0.5 W/W% of Wakogel 103 adjusted tp pH 6.0 (Wako Pure

Chemical Industories Co., Ltd.; carboxy vinyl polymer) to an amount of 100 g. Then, the mixture was filled into injection cylinders in aliquots each of 1 ml to provide intrarectal injection preparations.

Example 30

GlyOEt.HCl (29.31 g), Boc-Pro (43.05 g), HOBt (28.37 g) in DMF (150 ml) was treated with WSCD (38.43 ml) in an ice-bath, and stirred for 2 hrs. at 0°C, further overnight at room temperature. The reaction mixture was evaporated in vacuo. The residue was dissolved in 1 ℓ of AcOEt and washed with 5 % aqueous sodium bicarbonate solution, aqueous NaCl solution, 1-N HCl, 1-N NaOH and distilled water in this order. The solvent was evaporated in vacuo after dehydration to give a slightly yellowish oily product. (Boc-Pro-GlyOEt; 70.2 g).

The oily product in methylene chloride (20 ml) was treated with 70 ml of TFA at 0°C and stirred for 30 minutes at room temperature. The solvent was evaporated and resulting oily residue was added to NMM for neutralization at 0°C. Myristic acid (7.54 g) in THF (50 ml) was treated with WSCD (6.59 ml) at 0°C, then stirred for 1 hr. and added to above neutralized solution and stirred overnight at room temperature. This reaction mixture was concentrated and dissolved in chloroform (300 ml) and washed with 5 % sodium bicarbonate, 1N-HCl, 1N-NaOH and distilled water in this order. The solution was concentrated after dehydration with sodium sulfate and applied to Sephadex L-20 column (4 × 120 cm), and eluted with Benzene-AcOEt (1:1). The fraction was applied to TLC on silica gel by the following solvent system ($CHCl_3$:MeOH:AcOH 95:5:3), the part of Rf=0.30 was collected and extracted with $CHCl_3$. This solution was evaporated in vacuo and an oily product was obtained (N-myristoyl Pro-GlyOEt). Yield 76.8%.

The product was dissolved in EtOH (30 ml) and 1N-NaOH (33 ml) was added to the solution at 0°C. The resultant solution was stirred for 1 hrs. at room temperature and evaporated in vacuo. The concentrated solution was applied to a column of Sephadex L-20 (4 × 120 cm). The UV absorption at 230 nm was determined in each fraction (12.5 ml each). The fraction corresponding to the main peak (tube No. 95-111) were combined and freeze dried after concentration. White powders are obtained (N-Myristoyl Pro-GlyONa). Yield 56.6 %, m.p. 145-148°C. Rf=0.80 (n-Butanol: Acetic acid: H_2O 3:1:1). Amino acid ratio in a 6-N-HCl hydrolysate: Proline 0.90, Glycine 1.00

Calcd. for: C, 59.70; H, 9.31; N, 6.92
Found: C, 59.61; H, 9.21; N, 7.22

Example 31

Using GlyOMe·HCl (30.2 g), Boc-Proline (4.3 g), HOBt (28.37 g) and WSCD (38.43 ml), the process was carried out similarly as in Example 16, whereby slightly yellowish oily product was obtained (BOc-Pro-GlyOMe). The product (10.31 g) in dioxane (5 ml) was added to 4.32 N-HCl in dioxane (30 ml) in an ice bath, then stirred for 30 min. at room temperature. The solution was evaporated and drived in vacuo, thereafter dissolved in DMF (30 ml) and neutralized by addition of NMM (0.5 ml). Boc-Proline (6.46 g), HOBt (4.05 g) in DMF (30 ml) was treated with WSCD (5.49 ml) and stirred for 30 min. in an ice bath. The solution was added to above neutralized solution and stirred for 2 hrs. at 0°C and further overnight at room temperature. The solution was evaporated and dissolved in $CHCl_3$ (300 ml), then washed with 5 % aqueous sodium bicarbonate solution, 1N-HCl, 1N-NaOH in this order. The solution was dehydrated and concentrated to give an oily product (16.84 g) (Boc-Pro-Pro-GlyOMe). Rf=0.65 ($CHCl_3$: EtOH: AcOEt 5:2:5)

The product was acylated to N-Myristoyl Pro-Pro-GlyOMe (8.50 g) in the same manner as in Example 16. The oily substance dissolved in EtOH (30 ml) was added to 1N-NaOH solution (20.03 ml) and stirred for 30 min., then applied to Sephadex L-20 column. The main fraction was collected, concentrated and freeze dried to obtain white powders (4.61 g) (N-Myristoyl Pro-Pro-GlyONa).
Yield: 54.9%, Rf=0.66 (n-BuOH:AcOH:H_2O 3:1:1)

Calcd. for: C, 60.10; H, 8.92; N, 8.09
Found: C, 59.89; H, 9.20; N, 7.82

Amino acid ratio in a 6N-HCl hydrolysate: Proline 1.90, Glycine 1.00

Example 32

The process was carried out similarly as in Example 17, using capric acid instead of myristic acid, then N-Decanoyl Pro-Pro-GlyONa (7.22 g) was obtained. Yield: 81.0%, Rf=0.66 (n-BuOH:AcOH:H_2O 3:1:1)
Amino acid ratio in a 6N-HCl hydrolysate: Proline 1.8, Glycine 1.0

Calcd. for: C, 54.87; H, 8.38; N, 8.73
Found: C, 55.03, H. 8.62; N, 8.39

Example 33

The process was carried out similarly as in Example 17, using lauric acid instead of myristic acid, whereby N-lauroyl Pro-Pro-GlyONa (5.29 g) was obtained. Yield: 73.4 %, Rf=0.66 (n-BuOH:AcOH:H_2O 3:1:1)
Amino Acid ratio in a 6N-HCl hydrolysate: Proline 1.9, Glycine 1.0

Calcd. for: C, 58.64; H, 8.61; N, 8.55
Found: C, 59.01; H, 8.90; N, 8.20

CLAIMS

1. A preparation having excellent absorption property, comprising a medicine, a water-soluble substance at a concentration exhibiting higher osmotic pressure than isotonic sodium chloride solution, and one or more of N-acyl amino acid derivatives or N-acyl peptide derivatives represented by the formula: R-CO-A (wherein R is an aliphatic hydrocarbon group, an aromatic hydrocarbon group or an aryl-substituted lowerhydrocarbon group, which may optionally be substituted, and A is an amino acid residue or a peptide residue), as an absorption promoter.

2. A preparation according to claim 1, wherein the water-soluble substance at a concentration exhibiting higher osmotic pressure than isotonic sodium chloride solution is 1 W/W% or more of a water-soluble salt of an alkali metal.

3. A preparation according to claim 2, wherein the water-soluble salt of alkali metal is a halide, a sulfate, a phosphate or a carbonate of sodium, potassium or lithium.

4. A preparation according to claim 1, wherein the water-soluble substance at a concentration exhibiting higher osmotic pressure than isotonic sodium chloride solution is a 0.25 M or more of a water-soluble saccharide.

5. A preparation according to claim 4, wherein the water soluble saccharide is sorbitol, glucose, mannitol, lactose or sucrose.

6. A preparation according to any one of claims 1 to 5, wherein the content of the absorption promoter is 0.01 W/W% or higher of the whole content.

7. A preparation according to any one of claims 1 to 5, wherein the medicine is a water-soluble medicine having good water-solubility.

8. A preparation according to claim 7, wherein the water-soluble medicine has a partition coefficient of 50 or less in chloroform/water.

9. A preparation according to claim 1, substantially as hereinbefore described with reference to the Examples.

10. An absorption promoter, comprising a water-soluble substance at a concentration exhibiting higher osmotic pressure than isotonic sodium chloride solution and one or more of N-acyl amino acid derivatives or N-acyl peptide derivatives represented by the formula: R-CO-A (wherein R is an aliphatic hydrocarbon group, an aromatic hydrocarbon group or an aryl-substituted lower hydrocarbon group, which may optionally be substituted; and A is an amino acid residue or a peptide residue.

11. An aborption promoter according to claim 10, substantially as hereinbefore described with particular reference to the Examples.

Printed for Her Majesty's Stationery Office, by Croydon Printing Company Limited, Croydon, Surrey, 1982.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.